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THE ASCOCHYTA DISEASES OF PEAS AND VETCHES

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REPORT TO THE

GOVERNMENT OF ISRAEL

ON

THE ASCOCHYTA DISEASES OF PEAS AND VETCHES

By

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INTRODUCTION

In accordance with the request of the Government of Israel for technical assistance in the investigation of Ascochyta diseases of peas and vetches, the Food and Agriculture Organization of the United Nations, under its Expanded Technical Assistance Program, appointed Dr. J. de Tempe as Plant Pathologist, to advise and assist the Government in carrying out studies aiming at the elucidation of the taxonomy of the pathogens, the introduction of dependable tests for evaluation of seed infection, the initiation of trials for determination of control measures, and the formulation of a research plan for the future. The expert served in Israel from 11 July to 13 September 1959, during which period he worked mainly with the Faculty of Agriculture of the Hebrew University at Rehovoth.

It should be pointed out that the period during which the expert was in Israel was not the most suitable time of the year for the study of diseases of crops which in Israel are only grown in winter. Consequently, the expert had very limited opportunities to follow the development of crops and diseases in the field, or to conduct field and greenhouse experiments because of the high prevailing temperatures unfavorable to the temperate-climate crops. The possibilities for laboratory studies were also somewhat restricted, as the space and equipment available were rather insufficient for the required type of investigations.

In an effort to make better use of his time during the earlier months of his assignment, the expert somewhat broadened the scope of his activities, by assisting in the development of seed health studies in the Seed Research and Seed Testing Laboratory of the Agricultural Experiment Station at Bejt Dagon, and by lecturing on seed health and seed treatments as well as on the Ascochyta disease complex of peas in the Netherlands at both the Hebrew University and the Station.

Much useful information and material on Ascochyta diseases had been collected by plant pathologists in Israel, greatly facilitating the task of the expert. In this respect the expert is much indebted to Dr. I. Wahl, Lecturer in Plant Pathology at the Faculty of Agriculture, who, as the expert's counterpart, extended to him many courtesies. The expert is also grateful to Mr. I. Barash who, when studying for his master's degree, paid special attention to the Ascochyta diseases of peas, and who closely cooperated with the expert during his stay. The work done by Mr. Barash was of great benefit to the project.

The expert is much indebted to the officials and many members of the Faculty of Agriculture for the hospitality and assistance extended to him, to the Director and several staff members of the Agricultural Experiment Station for their courtesy and assistance, to the officials of the Plant Protection Service at Yaffo and the Government Agricultural Extension Service at Tel-Aviv for their active interest and cooperation, and to the personnel of the cooperative Hazera Ltd. at Haifa for their continuous help.

Special thanks are due to Dr. H. Gelmond of the Seed Research and Testing Laboratory, Mr. M. Bar-Daroma of the Agricultural Extension Service, Mr. J. Palti of the Agricultural Experiment Station, and Ir. S. Atsmon of the Mivhor Experimental Farm of Hazera Ltd. for their excellent cooperation and active interest. The first three enabled the expert to make several field trips which were useful even though the crops in the fields had not yet been sown or had scarcely emerged.

THE PROBLEM

Ascochyta diseases of peas are prevalent all over the world and have long been the subject of study in many countries. In recent years interest has somewhat abated, not because the problem has been sufficiently studied or the diseases can be effectively controlled, but because the possible lines of approach have been more or less explored without much progress having been made. However, special mention should be made of the work of the Canadian workers (Wallen et al) who are mainly concerned with Ascochyta pisi, particularly with respect to the development of resistant varieties of the host, and the work of the Eastern German group (Baumann, Sörgel, Stoll et al) who have made a thorough study of different aspects of the disease which in their region is mainly due to Ascochyta pinodella and Mycosphaerella (Ascochyta) pinodes.

In Israel peas are grown in large quantities and for various purposes, namely, as fresh vegetables and for canning, for fresh fodder, silage or hay, as green manure, and for seed production. Three main varieties are used, the garden pea Perfection and the field peas Dun and Hungarian. Perfection is an American variety of Pisum sativum, Dun an Australian variety of Pisum arvense, and Hungarian is of unknown origin but is probably a mixture of P. sativum and P. arvense which is badly in need of purification.

Climate and soil are very different in various parts of the country, influencing the selection of varieties as well as the purposes for which they are grown. Peas are grown exclusively in winter, developing on rains with eventual irrigation at the beginning of the season as in the case of early sowing for vegetable or fodder, or at the end of the season as in the case of late sowing for seed production. In general, one may say that the winter season normally is too cold and wet for this crop, so that in mid-winter it develops slowly and is often heavily stricken by diseases. In early winter, before the heavy rains start, there is scarcely any disease development, and the same is true again in late winter after the rains stop. These periods may permit the development of relatively disease-free seed. However, most of the sowing seed is imported. Often a pea field remains disease-free, or nearly so, throughout the season.

Vetches also are sown for different purposes, e.g.,as green manure, fodder and sometimes for seed production. Mainly the species Vicia atropurpurea and V. sativa are used, of which the former is more susceptible to Ascochyta infection. Most of the vetch seed is imported, chiefly from Sicily. Frequently vetch crops are totally destroyed by disease, although sometimes in a late stage new green sprouts may be formed and even seed produced. Certainly the financial losses caused by Ascochyta diseases of both peas and vetches are very great.

Before the expert's arrival in Israel, Mr. Barash had already isolated pathogenic fungi from diseased parts of pea plants. A dark perithecia-forming fungus appeared to be very prevalent all over the country. This must have been Mycosphaerella (Ascochyta) pinodes. Perithecia were found late in the season on plant parts which had totally died off. When the rain started in the autumn, new perithecia were formed on straw and stubble remaining in the field, and old perithecia again started producing ascospores. Late in the season Ascochyta pisi was also found and isolated.

Mycosphaerella isolates were used for cross-inoculation experiments in which were included a number of legumes commonly used in Israeli agriculture. The results of these experiments indicated that for practical purposes it is not necessary to take into consideration other host species than peas. Ascochyta pisi seems to have wider host range but, as already stated, it is of less importance on peas in Israel.

A beginning was made with the testing of pea varieties for susceptibility, mainly to Mycosphaerella pinodes. The expert's opinion is that it will be difficult to find or develop varietal resistance, although differences in tolerance certainly occur.

The situation, as outlined above, indicates several possible lines of approach which, however, could not be adequately pursued by the expert because of the season during which he was in the country, the limitation in available plant material such as straw and seed samples, and the inadequacy of laboratory equipment. Some of the possible lines of investigation were, and still are, as follows :

1. Determination of the identities of the Ascochyta species concerned through the use of straw and seed samples, and determination of the frequency and relative importance of seed transmission of the diseases by the use of seed samples.

2. Evaluation of seedling resistance in laboratory experiments, using single seedlings growing in glass tubes on nutritional solution.

3. Re-investigation of the effectiveness of field spraying in a normal growing season, using modern fungicides and techniques of application.

4. Comparison of fungicides for controlling seed-borne inoculum and for combating seedling damping-off due to soil fungi in greenhouse and field experiments.

5. Variety testing and in connection a program of breeding for resistance.

6. Cross-inoculations, including wild pea and vetch species.

7. Studies on the development of the crops and their diseases in the field, with special reference to factors which induce liberation of spores of the pathogens and factors which are necessary for successful infection of the hosts.

ACCOMPLISHMENTS

Investigation of Straw Samples

On most of the pea straw samples available, Mycosphaerella (Ascochyta) pinodes was present, in the majority of cases only the pycnidia of the fungus, with broadly ellipsoid and two-celled spores. In some cases, perithecia were also present, but they were found only in straw samples taken in the spring. Pycnidia of Ascochyta pisi were found in one out of eight samples. This species can be recognized by its more slender, two-celled pycnidiospores. This finding confirmed the impression that the latter fungus is of minor importance in peas in Israel.

In the ten vetch straw samples available, only the pycnidia with the rather coarse, two-celled spores of Ascochyta viciae were observed.

Additional pea and vetch straw samples were incubated in a moist chamber, either with or without previous surface disinfection, and after some days they were inspected for developing fungi. In addition to the pycnidia of Ascochyta species, acervuli of Colletotrichum species were also produced in some instances, in peas as well as in vetch. It is not certain that the Colletotrichum species found are pathogenic, and they were of small importance in the samples. The fungi concerned were isolated in order to test them in inoculation experiments as soon as weather conditions became suitable.

Investigation of Seed Samples

All pea and vetch seed samples which could be obtained, from the Seed Testing Laboratory or Hazera Ltd., were investigated with the moist blotter method for the presence of seed-borne pathogenic fungi. For routine investigation of commercial pea seed samples in the Netherlands, this method, with 5 days incubation at 20°C. in darkness, is quite satisfactory. For research purposes, however, the indicated duration appears to be insufficient and, especially when the individual seeds are only slightly infected, incubation up to 14 days at 20°C. is desirable in order to permit the infection to develop fully. Even with the length of time extended, nearly all the Israeli pea seed samples investigated seemed to be free from infection. If any infection was found, it was only in a low percentage and always with Mycosphaerella pinodes (Ascochyta stage). Only one sample, from a previous years experiment with early sowing in a field which had produced during the preceding year a heavily infected crop, showed about 40 percent seed-borne infection by M. pinodes.

It is very probable that pea seed produced under normal agricultural conditions in Israel, under a suitable crop rotation system, is usually not, or only slightly, infected, but that under exceptional conditions it may become heavily infected. It is also apparent, as indicated by the single sample mentioned, that soil-borne infection can play an important role, resulting in a heavily infected crop, which may produce heavily infected seed.

During the later months of the expert's stay, when room temperatures became moderate and the use of incubators was no longer essential for the blotter tests, a number of pea seed samples from a sowing-time experiment, conducted in the previous year by Hazera Ltd., were investigated. Plastic boxes with moist filter paper and translucent plastic covers were placed in the laboratory close to the window. Notwithstanding the fact that the plots from which these seed samples had been harvested had shown scarcely any disease, an appreciable amount of seed infection was found. The figures were as follows :

	Sowing Time				
	I	II	III	IV	
Seed sample A	18	10	6	5 %	Mycosphaerella pinodes
Seed sample B	9	6	8	2 %	"

These figures support the commonly held opinion that late sowing may result in partial disease escape of the new-formed seed. Moreover, they suggest that application of normal day-and-night alternation, or at least of sufficiently strong light, may result in a higher percentage of infection than that obtained in a blotter test in darkness. However, this impression is also in need of corroboration.

Another possibility for seed health testing is that of plating surface-disinfected seeds on nutrient agar (pea extract agar) and investigating the agar plates after a suitable time for the fungus colonies developed from the seeds. Again, for this method the commonly used 5 days' incubation is satisfactory for routine work but insufficient for research purpose. The method is attractive in that it saves incubator space and takes less time for accurate final inspection that does the blotter test. Its main disadvantage is that it requires absolute sterility whereas for the blotter method virtual sterility is sufficient.

Using a marrow fat pea sample obtained from the Netherlands, the following percentages of infection were obtained in 12 days at room temperature and with day light in the case of the blotter method :

	Mycosphaerella pinodes	Ascochyta pisi	Stemphylium botryosum
Blotter method	14	12	5 %
Agar method	11	8	4 %

The differences in results between the two methods might be within the standard error of the test and therefore not significant. A comparison of the methods should be made with other infected pea seed samples. The blotter-light method should be tried on a large number of Israeli and imported pea seed samples in order to estimate the extent of seed-borne infection in the sowing seed, and also to verify the view that the seed-borne infection is of relatively minor consequence. In this regard it should be remembered that Stemphylium botryosum is a weak pathogen which is injurious to peas only in the seedling stage.

The inspection of dry pea seed for Ascochyta spp. in ultraviolet light (Philips Philora lamp) appeared to be of value with heavily infected samples from the Netherlands, even though it certainly is not an accurate method. It seems to be of doubtful value for lightly infected Israeli pea seed since samples of Israeli origin that appeared suspicious in ultraviolet light usually appeared to be infection-free in subsequent blotter tests.

For vetch seed, the blotter test was first tried without finding any pycnidia-forming fungi in a number of samples, either imported or home-grown. Then the pea agar method was tried with a series of samples from seed lots recently bought in Sicily. Of the five samples tested, one was found to be infected to an extent of 44 percent. In the blotter test none of these samples showed any trace of infection. These findings agree with literature information, according to which the vetch seed coat is too hard and dry to permit Ascochyta development in a moist chamber test. Furthermore, of new samples from the same seed lots received a few weeks later, one in three was found to be heavily infected in the agar test (26 percent pycnidia-forming fungi). When the same method was used with three samples of locally-grown sativa vetch, infections of 34, 13 and 0 percent were found. Repeated testing of the same material made it clear that the method is far from reliable, although it is no doubt preferable to the blotter test for the kind of infection under discussion. It may be concluded, nevertheless, that Ascochyta infection is very prevalent in vetch seed.

From the preceding observations several conclusions may be tentatively drawn :

Peas. Mycosphaerella pinodes is predominant on peas in Israel. This fungus differs from the other Ascochyta species concerned in that it produces two types of spores, the slimy water-borne pycnidiospores of the Ascochyta stage and the dry wind-borne ascospores. Primary infection originates from either seed-borne inoculum or inoculum in infected plant debris remaining in the field. Secondary spread in the field may occur through dissemination of pycnidiospores by rain drops or by contacts between wet plants or through dissemination of ascospores. From the literature it is known that ascospores may be carried by the wind over distances of hundreds of meters, so the possibility of secondary infection cannot be eliminated, even when a good crop rotation has been practised.

The importance of inoculum of field origin was demonstrated in a sowing-time experiment. Disease-free seed was sown in a field which had produced a heavily infected crop the previous year. In the beginning of December, only one month after the first sowing, the young plants showed numerous lesions on the lower leaves but without any foot rot that might have originated from the seed.

Vetches. With vetches the situation seems to be quite different from that with peas. Ascochyta viciae, exclusively, was found in the straw and this fungus does not produce inoculum which is adapted for long-distance dissemination. Consequently, when a suitable crop rotation system is practised, an outbreak in the field can only arise from seed-borne inoculum. In fact, high percentages of seed infection were repeatedly found.

Laboratory Testing of Seedling Resistance

Experiments on resistance of pea seedling to Mycosphaerella pinodes were conducted in the laboratory with single seeds, either surface-disinfected with chlorine or alcohol or not disinfected, and placed on filter paper stools in glass tubes with nutrient solution or water. Everything was kept as sterilized as possible, and yet bacteria and a fungus of the Fusarium oxysporum type caused much trouble. Notwithstanding this difficulty, the results appear to indicate that certain varieties of Pisum arvense possess a limited degree of seedcoat resistance or tolerance to M. pinodes. The seedcoat of the commonly used variety Dun was frequently infected without showing any decay. However, in view of the facts that Dun may become heavily infected in the field, that its seedling resistance was often low, and that seed infection is rare in this variety, it was not considered worthwhile to continue these experiments.

Vetch seeds, either sound or artificially injured and inoculated with Ascochyta viciae did not produce seedlings with disease symptoms in experiments similar to those conducted with pea seeds. It is possible that seedling resistance is general in vetch but, if so, it remains to be found out how the seed-borne infection reaches the growing plant in the field.

Field Experiments

During the expert's stay in the country, a field experiment on seed treatment of peas and vetches was planned, using mainly commercial fungicidal mixtures containing an organic mercurial disinfectant and a protectant (thiram or COBH), because such preparations had proven most promising in laboratory experiments in the Netherlands. For the vetches an antibiotic was also included in the experiment because of the importance of seed-borne infection in this crop. The intention was to use this experiment primarily for emergence percentage countings but, in the case of vetches, also for determining the extent of disease development in the field and its effect on quality and quantity of seed yields. For the latter purpose the seed was to be sown late in the season, after the expert's departure.

For comparison, an experiment of similar design was conducted in the greenhouse as soon as temperatures were sufficiently low. The plantings in this experiment were not ready for emergence countings when the expert had to leave.

Two sowings of a pea sowing-time experiment were made in the field before the expert's departure. The intention was to make five sowings, to follow the extent of disease development in the field, and to determine the influence of sowing time on the new seed. In December the young plants of the first sowing had already developed the disease typically contracted from inoculum of field origin.

It was also the intention of the expert to conduct a field experiment on the effects of modern fungicides and spraying techniques on the development of the disease. However, as the officer of the Plant Protection Service who would have to do the spraying did not have confidence in a field experiment without laboratory experiments preceding, an arrangement was made with the Plant Protection Service to perform small-scale experiments with artificially infected pea plants in the greenhouse.

Field Observations

Field observations could not be actually made during the expert's stay in Israel. A questionnaire was prepared for the purpose of determining correlations between seed qualities of peas and environmental conditions on the one hand, and the extent of disease development in the field on the other. This questionnaire was discussed with Mr. M. Bar-Daroma, Chief of Field Crops of the Government's Extension Service, and explained in details to the field workers.

The need for a field survey is indicated particularly by the apparent dominating importance of air-borne infection and also by the sporadic nature of disease outbreaks. In order to determine the factors governing

the disease development, a survey as described in the questionnaire should include a large number of fields in all parts of the country and be extended over several years. The information thus collected must be carefully analyzed and collated in order to establish the relationship between the various factors involved. It will be necessary for the legume specialists of the Agricultural Experiment Station to take part in the survey, giving special attention to a limited number of pea fields.

A necessary complement to the field investigation will be studies of spore discharge and host plant infection in the field. A spore trap should be constructed for the purpose of correlating the presence of Mycosphaerella ascospores in the air with meteorological conditions. For studying host plant infection it will be necessary to use catching plants which are exposed to infection for short periods at different times of day or night. Afterwards, they should be incubated for two days at high humidity to allow the infections to develop. Such studies had been either planned or initiated by the expert before his departure and should be continued to obtain results.

MISCELLANEOUS OBSERVATIONS

Some attention was paid to legumious crops other than peas and vetch, i.e., alfalfa, clover, chick pea and fenugreek. From clover straw a Colletotrichum sp. was isolated, probably C. trifolii which causes the disease known as southern anthracnose. Seeds of other legumes were of little interest, although in alfalfa Stemphylium sarcinoforme and Ascochyta imperfecta were found.

During the summer, when the expert was not fully occupied by the Ascochyta project, he assisted in the development of seed health testing in the Seed Research and Seed Testing Laboratory of the Agricultural Experiment Station. Through this work it was observed that in general seed-borne pathogens are not as important in seed grown in Israel as in Western Europe. Still, some interesting observations were made. Helminthosporium teres was rather generally present in barley seed samples, but a local scientist observed that this seed-borne pathogen is of no practical consequence because of coleoptile resistance. In wheat, H. pedicellatum and H. tetramera were found. H. sativum is probably omniprosent in barley and wheat but apparently is seldom important. In a fungicide-treated lot of Clinton oats imported from the United States, H. victoriae was detected by a local worker. This fungus appeared only after three weeks in the blotter test, indicating the limited value of short-duration tests for treated seed. The lot of 1,000 kg. of infected seed was destroyed in order to prevent this pathogen from establishing itself in the country.

FUTURE ACTIVITIES

As previously stated, although certain lines for approaching the Ascochyta problem were provisionally laid down by the expert, not much could be completed or definitely established. For peas, Mycosphaerella pinodes is the most important pathogen. Seed transmission is of minor importance, and solution of the problem will have to be sought in the field. For vetch, Ascochyta viciae seems to be the sole species of practical importance and the disease is disseminated mainly by infected seed. The solution seems possible through laboratory investigations.

Research on Peas

The methods for testing infection in pea seed have been gradually improved. Different methods, e.g., the blotter test in darkness and in light, and the agar test in darkness and in light, should be compared. With the best method the prevalence of Ascochyta spp. in seed samples should be studied. Then the importance of seed infection at certain levels to the developing crop should be studied under inoculum-free field conditions, using a method similar to that which has been employed for certain cereal and flax diseases in the Netherlands. Because of the varying soil and weather conditions in Israel, this is a task which might well require many years.

In sowings with disease-free seed the importance of inoculum of field origin should be investigated.

For evaluation of the roles of seed-borne inoculum and inoculum of field origin, a survey will be important and, in addition, experiments should be conducted in the field. The field survey should be aimed at the determination of factors governing disease development in the field. It is also important to study the conditions that favor the liberation of ascospores and that favor the ascospore infection of the host.

A further step will be the study of varietal resistance, although this might be less fruitful in connection with Mycosphaerella pinodes than with the far less virulent Ascochyta pisi. This would be a long-range program in which wild pea species should be included as they may transmit the disease or bear genes for resistance. The possibility of perpetuating the disease by other leguminous crops requires further study by means of cross-inoculations.

An evaluation of the efficiency of modern spraying techniques and fungicides for controlling the disease should be carried out in the field.

The fact that Israel-grown pea seed is normally infected in low percentages and rather superficially offers an opportunity for fungicidal seed treatment, even though the slightly infected seed is not suited for experiments in this connection. On the other hand, as pea seed should always be treated before sowing as a precaution against damping-off caused by soil fungi, the possibility of controlling seed-borne disease by such treatments should also be considered. To this end, field trials will be necessary to determine the effective treatments.

As the program for investigating the pea disease is a complicated one, it is essential that the plant pathologist in the Faculty of Agriculture be designated to establish working relationship with other institutions and persons engaged in studies of this disease, and to assume the responsibility of organizing and co-ordinating the work.

Research on Vetches

For vetch the first requirement is the development of a reliable method of seed health testing, to be followed by seed treatment studies which should be conducted in the field in disease-free surroundings. With the establishment of a reliable seed health-testing method, care can be exercised to avoid the purchase or sowing of heavily infected seed.

RECOMMENDATIONS

For the tasks indicated in the preceding section the present equipment of the Plant Pathology Laboratory of the Faculty of Agriculture is certainly insufficient. For testing seed samples and comparing fungicide treatments, a reliable and spacious conditioned germinator or germination room is a necessity. It should be capable of being maintained at a reasonably uniform temperature of about 20°C. without strong ventilation, and should contain uniform and sufficiently strong illumination provided by fluorescent lights.

For preliminary soil experiments a greenhouse is now available, but it cannot be used during the greater part of the year because of the high temperatures. The length of the period during which soil experiments might be undertaken could be extended a few months by building shaded open-air benches with gauze protection against birds. These could be used either for pot experiments, with the pots sunken into a moist medium such as peat dust, or for experiments conducted directly in the bench soil.

A spore trap should be constructed for use in making field observations.

Notwithstanding good co-operation with other institutions, the tasks to be performed by the Faculty of Agriculture will soon be beyond the capacity of a single man. It would then be advisable to provide him, at least during the winter season, with student assistants who could carry out laboratory work under his supervision.